Established in 1871
Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Letter to the editor | Published 18 October 2011, doi:10.4414/smw.2011.13278
Cite this as: Swiss Med Wkly. 2011;141:w13278

Letter to the editor

Assessing the role of platelet activation in bevacizumab associated thrombosis

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In a recent issue of Swiss Medical Weekly [1], Fehr and colleagues report findings from a study designed to address the clinical relevance of a molecular mechanism we suggested may link bevacizumab (bev) to thrombosis [2]. However, we would respectfully point out that the data presented by Fehr et al. do not support their conclusion that our proposed mechanism cannot be "a major contributing factor" to bev-associated thrombosis. In our study, we showed that when bev and $VEGF_{165}$ were combined in balanced stoichiometry, the resulting higher order immune complexes (ICs) activated the IgG receptor, FcγRIIa, both in human platelets and in mice transgenic for human FcγRIIa [2]. We identified heparin as a vital component of this mechanism, as $VEGF_{165}$ is a heparin-binding protein [3]. Although the authors state their study was not designed to assess the impact of heparin in bev-treated patients, the study objective (to test the clinical relevance of our proposed mechanism) should have led the authors to design their experiments with attention to the heparin-binding capacity of $VEGF_{165}$. However, Fehr et al. used a type of VEGF predicted to lack heparin binding activity. Santa Cruz Biotechnology (SCBT) "VEGF (hBA-165)" (sc-4570) has an amino acid sequence "deletion at 141-208" [4], which removes the heparin-binding domain [3]. It is doubtful this type of VEGF could support the heparin-dependent activity we originally described [2]. This matter aside, the authors tested bev and VEGF alone and in combination, but not in the presence of heparin. We have already shown that under these conditions, bev typically lacks platelet activating activity [2]. Fehr and colleagues combined 500 µg/milliliter bev with 500 µg/liter VEGF, resulting in a molar stoichiometric imbalance far outside the zone of equivalence (i.e. >100-fold less VEGF than bev). These conditions do not favour the formation of higher order ICs, which are required for FcγRIIa signaling. Indeed, we have previously determined that stoichiometric imbalances of this magnitude render bev+VEGF inactive, even in the presence of heparin (unpublished data).

Fehr et al. used the PFA-100 instrument to test the effect of bev+VEGF on platelet function. Citing several reports, the authors contend that, although the PFA-100 was specifically designed to detect platelet dysfunction, "it is also capable of detecting an increased adhesive and aggregatory platelet function." Such enhanced platelet function would be reflected in shortened PFA "closure time" (CT) values, which were not observed with bev±VEGF. However, the "question of whether shortened PFA-100 CTs per se might also reflect a prothrombotic tendency" remains a topic of ongoing investigation [5]. The PFA-100 has not been clinically validated for such use, and its design principle suggests it would not be a sensitive measure of thrombotic risk, since blood coagulation is blocked in the system by citrate, and since CT is controlled by collagen plus epinephrine or ADP. Indeed, preincubation of blood with bev plus little or no VEGF and no heparin can hardly be expected to add significantly to the potent activation stimuli of collagen plus epinephrine or ADP, which together rank among the most potent of platelet agonists.

If bev-induced platelet activation contributes to thrombosis in cancer patients, we suggested the process would not be rapid, due to limited VEGF availability [2]. A physiologically plausible way that our proposed mechanism could contribute to thrombosis would be through the gradual accumulation of bev+VEGF complexes at sites of vascular disturbance, leading to micro-thromboembolism [6]. In this view, bev+VEGF-activated platelets would be removed from circulation, much as in the case of heparin-induced thrombocytopenia (HIT). Because of this, we would not expect to find any profound changes in systemic markers of primary or secondary haemostasis due to bev therapy, beyond those already linked to cancer and chemotherapy. Interestingly, this was the case in a study by Gerber and colleagues, who subjected mice lacking FcγRIIa to prolonged high dose bev therapy and were unable to demonstrate systemic haemostatic dysfunction [7]. In an effort to identify systemic platelet activation in bev recipients, Fehr et al. measured serum soluble P-selectin (sPsel) levels. However, measurement of sPsel in serum rather than plasma merely reflects complete release of the molecule from all platelets, which are activated during the production of serum [8]. Therefore, the significance of the sPsel values obtained is unclear.

For these reasons, we believe the work of Fehr et al. fails to exclude the possibility that bev-associated platelet activ-

ation via FcγRIIa may contribute to thrombosis in cancer patients.

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